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Laboratory-based Surveillance Detects Listeriosis Outbreak

by Harvey George

Once again, submission of clinical specimens from hospital laboratories to state public health laboratories has aided the detection of a putative common source outbreak. A multi-state outbreak of a single strain of *Listeria monocytogenes* (LM) with 41 reported cases as of December 25, 1998 has been identified in 10 states. The reported cases have occurred in Massachusetts (4), Ohio (13), New York (12), Tennessee and West Virginia (three each), Michigan (2), and Connecticut, Georgia, Oregon and Vermont (one each). Dates of onset of illness or isolation of LM ranged from August 2 through December 2. Four of the 41 cases have been fatal, and one of the fatal cases was in Massachusetts. The LM isolates from this outbreak are serotype 4b and share an unusual PFGE or ribotyping subtype pattern. The pattern is a rare pattern among humans.

CDC, in collaboration with health departments in Connecticut, New York, Ohio and Tennessee conducted a case-control study, comparing 4-week food histories of 20 patients infected with the outbreak strain with the food histories of 20 control patients infected with other LM strains. Sixteen (89%) of 18 cases, but only six (32%) of 19 controls, consumed cooked hot dogs during the month before illness onset (odds ratio=17.3; 95% confidence interval=2.4-160.0; p less than 0.01). On December 19, the outbreak strain of LM was isolated from an open package of hot dogs that a patient had eaten 4 weeks before onset of listeriosis. On December 22, Bil Mar Foods, the manufacturer of the hot dogs, voluntarily recalled specific production lots.

Listeria monocytogenes, a gram-positive rod shaped bacterium, is not usually considered pathogenic to the general population. Typically, a person who acquires infection exhibits an acute, mild, febrile illness, sometimes with influenza-like symptoms. The individuals at highest risk to contract the disease are neonates, the elderly, immunocompromised individuals and pregnant women. Listeriosis is usually manifested as meningoencephalitis and/or septicemia in newborns and other more susceptible populations. Infection may be dangerous in pregnant women because transfer of the infection may occur to the fetus. Even though a mother is asymptomatic, infections transferred to the fetus may result in stillbirths, septicemia in a newborn, or development of meningitis in the neonatal period.

Outbreaks of listeriosis have been associated with ingestion of raw or contaminated milk, soft cheeses, contaminated vegetables and ready-to-eat meats. A substantial proportion of sporadic cases of listeriosis probably results from foodborne transmission. The incubation period is variable, with documentation of symptoms and infection occurring from 3 to 70 days following exposure.

All *Listeria monocytogenes* isolates should be sent to the State Laboratory Institute, and all cases of listeriosis should be reported to the Massachusetts Communicable Disease Control Bureau. SLI can subtype LM by PFGE methods to identify associations among cases and foods.

Reference: MMWR, December 25, 1998/47(50);1085-6.

Genetic Testing Recommendations by CLIAC

by Dina Caloggero

Currently, clinical laboratories that perform genetic testing are regulated under CLIA '88. However, the Department of Health and Human Services (HHS) felt that, due to the nature of genetic testing, additional requirements specifically for genetic testing might be needed. On September 16-17, 1998, the Clinical Laboratory Advisory Committee (CLIAC) met with its Genetic Testing Workgroup (GTS) and made final recommendations to the CDC requiring further regulations to CLIA '88 specifically for genetic testing. Please note that CLIAC is an advisory panel and its views are not binding on the CDC.

In constructing the additional recommendations, the GTS first developed a definition for genetic testing and then examined it through all phases of testing. The subspecialty areas identified and defined were

"Molecular genetic and cytogenetic test - The analysis of human DNA, RNA, and chromosomes to detect heritable or acquired disease-related genotypes, mutations, phenotypes, or karyotypes for clinical

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purposes. Such purposes include predicting risk of disease, identifying carriers, and establishing prenatal or clinical diagnoses or prognoses in individual families or populations."

"Biochemical genetic test - The analysis of materials derived from the human body, including human proteins and certain metabolites, predominantly used to detect inborn errors of metabolism, heritable genotypes, or mutations for clinical purposes. Such purposes include predicting risk of disease, identifying carriers, and establishing prenatal or clinical diagnoses or prognoses in individual, families, or populations. [Tests that are used primarily for other purposes, but may contribute to diagnosing a genetic disease (e.g., blood smear, certain serum chemistries) would not be covered by this definition.]"

CLIAC recommends that many of the current standards that apply to non-genetic testing also apply to genetic testing. A summary of some notable regulations specifically proposed for genetic testing follows.

Re-use of Tested Specimens - If the laboratory intends to use previously tested samples for QC and QA purposes, it should a) remove all patient identifiers or obtain patient consent and b) have a procedure in place that permits patients to elect not to have their specimens used for these purposes, even if the laboratory intends to remove all patient identifiers.

Personnel Qualifications - Within the genetic testing specialty and/or all laboratory specialties under CLIA '88, the Technical Supervisor should be upgraded to Technical Director. CLIAC felt that this would reflect a "real world" situation due to the position's level of responsibilities. The Clinical Consultant and General Supervisor levels should both require specific genetic testing experience, but not necessarily in the relevant subspecialty.

Personnel Responsibilities - The Technical Supervisor (Technical Director) should ensure that reports include pertinent information required for specific genetic testing. The Clinical Consultant should assist providers in ordering tests to meet appropriate clinical needs.

Specific Quality Control/ Contamination - The laboratory should be designed to minimize and control contamination when performing nucleic acid amplification procedures. Work processes should minimize the risk of mixing samples and contaminating equipment, reagents or samples. RNA work areas must be separated from DNA work areas.

Specimen Integrity - To ensure proper identification of the subject being tested, specimens should include a unique identifier, DOB, gender, ethnicity, patient or family number, and laboratory number; the specimen source; the date/time of collection and arrival in the laboratory; and the name of the person who obtained the sample.

Validation of Tests - The following clinical parameters are proposed for genetic test validation: a) A positive confirmatory test should have a defined positive predictive value that can be communicated to the care giver; b) Where the disease prevalence is more frequent than 1/10,000, the validity should be documented in at least 10 positive probands (including cell lines or DNA/RNA) prior to offering the test; c) Predictive value should be defined in terms of ethnic populations, when applicable.

Special Reporting Requirements - Laboratories should ensure that results are understandable by a non-geneticist health care provider. They must include the following if applicable: interpretation, comments, recommendations for further testing or clinical consultation, and a summary of the method and its limitations. Specific requirements for reporting molecular genetic testing include a list of the mutant alleles tested; the detectability rate of the panel; a revised risk assessment based on test results, as applicable; important clinical implications for other family members, as applicable; variables that affect test interpretation (e.g., ethnicity); and limitations of the testing.

Additional recommendations were made on issues of informed consent, appropriateness of tests, ordering additional tests, confidentiality as it relates to test result reporting, specific QC, proficiency testing, and record/specimen retention.

Reference: Summary Report from CLIAC Meeting 9/16-17/98, HHS, CDC: Summary of CLIAC Recommendations for Genetic Testing, Addendum D.

Grants, Projects & Publications

Laboratory Surveillance for Pertussis

by Harvey George

The State Laboratory Institute (SLI) has received funding from the CDC, as part of the Bureau of Communicable Disease Control's Immunization Cooperative Agreement, for an "Enhanced Pertussis Surveillance" project. SLI performs conventional culture isolation and identification of *Bordetella pertussis*, as well as a single serum diagnostic assay. The serologic assay is an ELISA IgG test for pertussis toxin. Unlike most tests that require acute and convalescent sera to diagnose a recent pertussis infection, this test can provide a diagnostic result with a single sample.

The project funds an initiative to identify the occurrence of specific pertussis strain types, and to distinguish outbreak strains from strains that are circulating in the general community. SLI is one of four laboratories that will participate in a national validation project to establish a standard method for the pulsed-field gel electrophoresis (PFGE) of *Bordetella pertussis*.

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Program Reports — Antibiotic Resistance Monitoring

by Joseph Peppe

Several years ago the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) launched the National Antimicrobial Resistance Monitoring System (NARMS). This program was designed as a collaboration between the CDC and fourteen (14) state and local health departments (CA, CO, CT, FL GA, KA, Los Angeles County, MN, MA, NJ, New York City, OR, WA, and WV). Other states have since joined.

One of the primary goals of this project is to prospectively monitor the prevalence of antimicrobial resistance among human non-typhoidal *Salmonella* and *Escherichia coli* O157:H7 isolates. Each participating site, after completing identification and / or confirmation and serotyping, submits every tenth *Salmonella* isolate and every fifth *Escherichia coli* O157:H7 isolate to the CDC for susceptibility testing. CDC screens each isolate with a panel of seventeen (17) antimicrobial agents to determine their minimum inhibitory concentrations (MIC). The seventeen antimicrobials are amikacin, ampicillin, amoxicillin-clavulanic acid, apramycin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, trimethoprim-sulfamethoxazole, and ticarcillin.

To determine the susceptibility of the isolates, CDC employs a semi-automated system (Sensititre, Accumed, Westlake, OH). Campylobacter was added to the list of organisms tested and eight states (CA, CT, GA, MD, MN, NY, OR, TN) have submitted isolates since 1997.

The total number of *Salmonella* isolates submitted to the CDC during calendar year 1997 was 1,314. Ninety-nine percent (1,301) of those submitted were tested for antimicrobial susceptibility. The Massachusetts State Laboratory Institute (SLI) submitted 9.8% (129) of the total number of isolates. CDC tested a total of 161 *Escherichia coli* O157. Of these, 15.5% (25) were isolates submitted by the SLI.

Forty of the 129 *Salmonella* isolates from MA were *Salmonella typhimurium*. Susceptibility testing revealed that fourteen (35%) were penta-resistant. This resistance pattern, referred to as R-type ACSSuT, includes ampicillin, chloramphenicol, strepomycin, sulfonamide and tetracycline. Three points of interest are as follows: (1) *Salmonella typhimurium* with this resistance pattern were more commonly isolated from blood than other *S. typhimurium* and other *Salmonella spp.* (2) This R-type strain has increased in frequency from 0.6% of the isolates in the late 1970's to 34% in 1996. (3) This pattern is indicative of the antibiogram typical of *Salmonella typhimurium* Definitive Type (DT)104. There is a high correlation between the strains demonstrating this penta-resistance and the DT104 strain. Phage typing performed at the CDC is necessary to confirm that penta-resistant strains of *Salmonella typhimurium* are in fact *Salmonella typhimurium* DT104.

Salmonella typhimurium DT104 has been recognized as an emerging foodborne pathogen. It is resistant to multiple antimicrobial agents, frequently demonstrating the penta-resistance pattern R-type ACSSuT. It is frequently associated with more severe illness than other non-typhoidal *Salmonella*.

Beginning on January 1, 1999, the SLI will submit all *Salmonella typhi* and every tenth *Shigella* isolate to the CDC for antimicrobial susceptibility testing. Please continue to submit all of your enteric isolates, so that we may continue to detect local and regional clusters and outbreaks. This also allows the SLI to continue to support the NARMS program in its attempt to follow national trends.

References: NARMS 1997 Annual Report, CDC; Michigan Department of Community Health, LabLink, Vol. 3 No. 2, November 1997 and Vol. 3 No. 3, April 1998.

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Previous collaborative investigations by our laboratory and the CDC have identified the presence of six *B. pertussis* strains during a recent high school outbreak, with two strains comprising the majority of the organisms associated with illness. The current project will determine PFGE patterns and establish a repository for *B. pertussis* isolates in Massachusetts. In addition, because of the recent reports of the emergence of two strains of erythromycin resistant *B. pertussis*, SLI will test all isolates for resistance to erythromycin. Erythromycin is prescribed generally for prophylaxis following pertussis exposure.

Data from the Massachusetts project will be combined at CDC with data from the collaborative projects (AZ, GA, IL, MA, MN, NY). The study data include PFGE patterns, erythromycin sensitivity (both Kirby-Bauer and E-test) and demographic and clinical information. These data will provide a national picture of the circulating strains of pertussis. In addition, they may provide insight on the efficacy of the acellular pertussis vaccines now in use and track erythromycin resistance in *B. pertussis*.

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for the electronic version of this newsletter and other information about the State Laboratory Institute: www.state.ma.us/dph/sli.htm.

Laboratory Training Activities

Internet Training: Shiga-toxin producing *E. coli* - Participate in a two-hour interactive learning program and earn CEUs on the Web! It's a fun and easy way to learn. Sign on at http://www.shore.net/~nacmid/ or call (617) 983-6285.

New Developments in Cystic Fibrosis - March 2, Holiday Inn Crowne Plaza, Rt. 9, Natick, MA. This one-day symposium will provide a comprehensive review of the pathogenesis and laboratory and clinical management of cystic fibrosis. Call (617) 983-6285.

Anaerobic Bacteriology for the Clinical Laboratory - April 8, State Laboratory Institute, Boston, MA. This one-day, intermediate-level workshop provides hands-on training in anaerobic bacteriology. Call (617) 983-6285.

State Laboratory Training Coordinator, *Garry R. Greer, BS, (617) 983-6608, E-mail:* garry.greer@state.ma.us
For a list of NLTN courses in your area sign on to the Web at http://www.cdc.gov/phppo/dls/nltn.htm.

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